

CLAIMS

1. Process for the isolation in aqueous phase of a
5 nucleic material present in a sample, comprising a step
of adsorption of said nucleic material onto a
particulate support, ~~wherein~~ ~~characterized in that:~~

* according to a ~~so-called~~ step (a) for
producing the adsorption reagent, an adsorption reagent
10 is available which comprises a sol consisting of an
aqueous continuous phase and a discontinuous phase of
the particulate support which comprises a
functionalized, particulate polymer, said polymer being
obtained by polymerization of (1) a first water-soluble
15 monomer of acrylamide or of an acrylamide derivative,
(2) at least one cross-linking agent and (3) at least a
second cationic and water-soluble functional monomer,
said polymer having a predetermined lower critical
solubility temperature (LCST) which is between 25 and
20 45°C,

* according to a ~~so-called~~ step (b) for
bringing into contact, the adsorption reagent is
brought into contact with the sample containing the
nucleic material,

* according to a ~~so-called~~ adsorption step (c),
25 for the bringing into contact according to (b), at
least one of the following parameters for the reaction
medium is chosen:

- pH at most equal to 7,
30 - ionic strength at most equal to 10^{-2} M,
- temperature less than the LCST of the poly-
mer,

* according to a ~~so-called~~ separation step (d),
35 after having optionally observed that the adsorption
has taken place, the discontinuous phase is separated
from the continuous phase, and

* according to a ~~so-called~~ desorption step (e),
the nucleic material is dissociated, by desorption,

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~~from the particulate support by increasing the ionic strength up to an ionic strength greater than 10^{-2} M.~~

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2. Process according to Claim 1, ~~characterized in that~~ for the desorption step (e), at least one of the 5 parameters selected from the pH and the temperature is in addition varied as follows:

- increase in the pH up to a pH greater than 7,
- increase in the temperature up to a temperature greater than the LCST of the polymer.

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10 3. Process for the isolation in aqueous phase of a nucleic material present in a sample, comprising a step of adsorption of said nucleic material onto a particulate support, ~~characterized in that:~~

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15 * according to a ~~so-called~~ step (a) for producing the adsorption reagent, an adsorption reagent is available which comprises a sol consisting of an aqueous continuous phase and a discontinuous phase of the particulate support which comprises a functionalized, particulate polymer, said polymer being obtained by polymerization of (1) a first water-soluble monomer of acrylamide or of an acrylamide derivative, (2) at least one cross-linking agent and (3) at least a second cationic and water-soluble functional monomer, said polymer having a predetermined lower critical 20 solubility temperature (LCST) which is between 25 and 45°C,

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25 * according to a ~~so-called~~ step (b) for bringing into contact, the adsorption reagent is brought into contact with the sample containing the 30 nucleic material,

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35 * according to a ~~so-called~~ adsorption step (c), for the bringing into contact according to (b), an ionic strength at most equal to 10^{-2} M is selected for the reaction medium,

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35 * according to a ~~so-called~~ separation step (d), after having optionally observed that the adsorption has taken place, the discontinuous phase is separated from the continuous phase.

4. Process according to Claim 3, ~~characterized in~~ *wherein* that, according to the adsorption step (c), for the bringing into contact according to (b), at least one of the following parameters is in addition selected for the reaction medium:

- pH at most equal to 7,
- temperature less than the LCST of the polymer.

5. *wherein* Process according to ~~any one of Claims 1 to 4~~, 10 ~~characterized in that~~ the particulate support consists of a functionalized particulate polymer obtained by polymerization of (1) a first water-soluble monomer of acrylamide or of an acrylamide derivative, (2) at least one water-soluble cross-linking agent and (3) at least 15 a second cationic and water-soluble functional monomer, said polymer having a predetermined lower critical solubility temperature (LCST) which is between 25 and 45°C, preferably between 30 and 40°C.

6. *wherein* Process according to ~~any one of Claims 1 to 4~~, 20 ~~characterized in that~~ the particulate support comprises, in addition, an organic or inorganic core, completely or partially coated with said particulate polymer, said core not modifying the adsorption properties of the polymer in relation to said nucleic 25 material.

7. Process according to Claim 6, ~~characterized in~~ *wherein* that the core is a polystyrene core.

8. *wherein* Process according to Claim 6 ~~or 7~~, 30 ~~characterized in that~~ the core comprises a magnetic compound.

9. *wherein* Process according to ~~any one of the preceding~~ ~~claims~~, ~~characterized in that~~ at least one probe and/or one primer capable of specifically hybridizing to the nucleic material is added to the sample before step (b), or to the reaction medium after step (b), and in particular after step (c) or step (d).

10. *wherein* Process according to ~~any one of Claims 1 to 8~~, ~~characterized in that~~:

* according to (b) and (c), the adsorption reagent is brought into contact with the nucleic material consisting of a probe or a ~~primerr~~ ^{primerv} in order to obtain a hybridization reagent,

5 * according to (b'), after having optionally observed that the adsorption has taken place, and separated the hybridization reagent from the reaction medium, said hybridization reagent is brought into contact with a medium containing at least one nucleic acid or nucleic acid fragment, under suitable 10 conditions for the hybridization or the extension of the primerv.

15 11. Process according to ~~any one of the preceding~~ ^{Claim 1} ~~claims, wherein~~ ¹ characterized in that the LCST of the polymer is between 30 and 40°C.

12. Process according to ~~any one of the preceding~~ ^{Claim 1} ~~claims, wherein~~ ¹ characterized in that the first monomer (1) is selected from N-alkylacrylamides and N,N-dialkylacrylamides.

20 13. Process according to Claim 12, characterized in that the first monomer (1) is selected from N-isopropylacrylamide, N-ethylmethacrylamide, N-n-propylacrylamide, N-n-propylmethacrylamide, N-isopropylmethacrylamide, N-cyclopropylacrylamide, 25 N,N-diethylacrylamide, N-methyl-N-isopropylacrylamide, N-methyl-N-n-propylacrylamide, the first monomer being preferably N-isopropylacrylamide (NIPAM).

30 14. Process according to ~~any one of the preceding~~ ^{Claim 1} ~~claims, wherein~~ ¹ characterized in that the second functional monomer(s) (3) are selected from the ~~acrylic~~ ^{acrylic} and methacrylic derivatives, 2-aminoethylmethacrylate chloride (AEM), ~~the~~ N-vinylpyridine derivatives, ~~the~~ trialkylammonium derivatives and ~~the~~ isothiouronium chloride derivatives.

35 15. Process according to ~~any one of the preceding~~ ^{Claim 1} ~~claims, wherein~~ ¹ characterized in that the water-soluble cross-linking agent (2) is selected from N,N-methylenebisacrylamide (MBA) ^{OR} ethylene glycol dimethacrylate.

16. Process according to ~~any one of the preceding~~
~~claims, characterized in that~~ the polymerization
initiator is selected from the water-soluble neutral
and cationic initiators ~~such as~~ ~~2,2'~~
5 ~~azobisisamidinopropane chloride (V50).~~

17. Process according to Claim 3, ~~characterized in~~
~~wherein~~ ~~that~~ it comprises, after the separation step (d), a ~~so-~~
~~called~~ desorption step according to which the nucleic
material is dissociated, by desorption, from the
10 particulate support by varying at least one of the ~~group consisting of~~ ~~the~~ pH and
~~the~~ temperature, as follows:

- increase in the ionic strength up to an ionic strength greater than $10^{-2}M$,
- 15 - increase in the pH up to a pH greater than 7,
- increase in the temperature up to a temperature greater than the LCST of the polymer.

18. Process according to ~~any one of the preceding~~
~~claims, characterized in that~~ the separation step (d)
20 is performed by a technique selected from centrifugation, filtration, precipitation, sedimentation, and the application of a magnetic field.

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